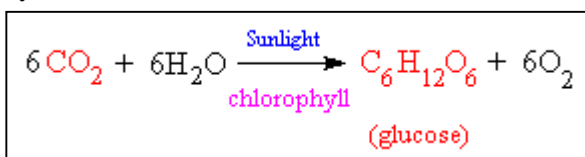


THE YSI CHLOROPHYLL TECHNOLOGY

Rick Fielder, YSI, Inc.

WHAT IS CHLOROPHYLL?

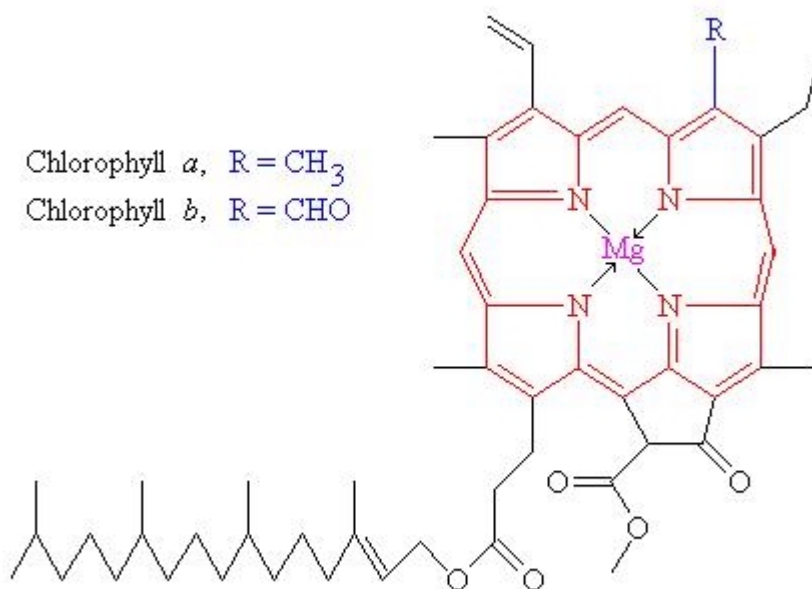
Chlorophyll, in various forms, is bound within the living cells of algae and other phytoplankton found in surface water. Chlorophyll is a key biochemical component in the molecular apparatus that is responsible for photosynthesis, the critical process in which the energy from sunlight is used to produce life-sustaining oxygen. In the photosynthetic reaction, carbon dioxide is reduced by water, and chlorophyll assists this transfer.



Photosynthesis

Chlorophyll is present in many organisms including algae and some species of bacteria. Chlorophyll *a* is the most abundant form of chlorophyll within photosynthetic organisms and, for the most part, gives plants their green color. However, there are other forms of chlorophyll, coded *b*, *c*, and *d*, which augment the overall fluorescent signal. These types of chlorophyll, including chlorophyll *a*, can be present in all photosynthetic organisms but vary in concentrations.

Chlorophyll enables plants and other chlorophyll-containing organisms to perform photosynthesis. Chlorophyll is a chelate, or a central metal ion, in this case magnesium, which is bonded to a larger organic molecule, called a porphyrin. The porphyrin molecule is composed of carbon, hydrogen, and other elements such as nitrogen and oxygen. The magnesium ion bonded within this ring is thought to be responsible for electron transfer during photosynthesis. (See structure below).



HOW IS CHLOROPHYLL MEASURED?

There are various techniques to measure chlorophyll, including spectrophotometry, high performance liquid chromatography (HPLC), and fluorometry. All of these methods are published in *Standard Methods for the Examination of Water and Wastewater*, 19th Edition.

Spectrophotometry is the classical method of determining the quantity of chlorophyll in surface water. It involves the collection of a fairly large water sample, filtration of the sample to concentrate the chlorophyll-containing organisms, mechanical rupturing of the collected cells, and extraction of the chlorophyll from the disrupted cells into the organic solvent acetone. The extract is then analyzed by either a spectrophotometric method (absorbance or fluorescence), using the known optical properties of chlorophyll, or by HPLC. This general method, detailed in Section 10200 H. of *Standard Methods*, has been shown to be accurate in multiple tests and applications and is the procedure generally accepted for reporting in scientific literature (see Reference section). The fluorometric method also requires the same extraction methods used with spectrophotometry, then uses a fluorometer to measure discrete molecular chlorophyll fluorescence. However, these methods have significant disadvantages. They are time-consuming and usually require an experienced, efficient analyst to generate consistently accurate and reproducible results. In addition, they do not lend themselves readily to continuous monitoring of chlorophyll (and thus phytoplankton) since the collection of samples at reasonable time intervals, e.g., every hour, would be extremely time-consuming.

YSI has developed a sensor for chlorophyll determinations both in spot sampling and in continuous monitoring applications. It is based on an alternative method for the measurement of chlorophyll which overcomes these disadvantages, albeit with the potential loss of accuracy. In this procedure, chlorophyll is determined *in situ* without disrupting the cells as in the extractive analysis. The YSI 6025 chlorophyll sensor is designed for these *in-situ* applications, and its use allows the facile collection of large quantities of chlorophyll data in either spot sampling or continuous monitoring applications.

It is important to remember, however, that the results of *in-situ* analysis will not be as accurate as results from the certified extractive analysis procedure. The limitations of the *in-situ* method should be carefully considered before making chlorophyll determinations with your YSI sonde and sensor. Some sources of inaccuracy can be minimized by combining extractive analysis of a few samples during a sampling or monitoring study with the YSI sensor data. The *in-situ* studies will never replace the standard procedure. The estimates of chlorophyll concentration from the easy-to-use YSI chlorophyll system are designed to complement the more accurate, but more difficult to obtain, results from more traditional methods of chlorophyll determination.